

Please delete the Sequence Listing section appearing at pages 38-62 of the application as filed, and append the substitute Sequence Listing submitted herewith at the end of the application. The substitute Sequence Listing is also submitted herewith in computer readable form.

#### **REMARKS**

The specification has been amended to add SEQ ID numbers to specific nucleic acid sequences referred to in the specification, in compliance with 37 CFR §1.821(b). The specification has been further amended to replace the Sequence Listing section originally appearing at pages 38-62 of the application with a substitute Sequence Listing, which is requested to be inserted at the end of the application. Separate sheets marked to show the amendments to the specification at pages 35 and 36 are attached.

The substitute Sequence Listing sets forth the sequences referred to in the specification and the sequences of the original Sequence Listing section together. The original sequences, as a result of being incorporated together in a new PatentIn 3.1 Sequence Listing, have been renumbered, however the sequence information has not changed, and no new matter has been added. Support for the amendments is apparent from the specification as filed at pages 25 (line 11), 29 (Table 4), 31 (line 5), 35 (lines 8, 25, 26, 29, 30), 36 (lines 5-6), and 38-62. A computer readable form of the substitute Sequence Listing is also provided herewith.

A statement under 37 CFR §1.825 accompanying a computer readable form of a copy of the Sequence Listing, and an executed oath or declaration are submitted concurrently in order to respond to the Notice of Missing Parts issued January 18, 2002.

Examination and allowance of the claims as amended herein are respectfully solicited.

Respectfully submitted,



Leon R. Yankwich, Reg. No. 30,237  
Regina S. Bautista, Reg. No. 46,280  
Attorney for Applicants  
YANKWICH & ASSOCIATES  
130 Bishop Allen Drive  
Cambridge, Massachusetts 02139  
telephone: 617-491-4343  
telecopier: 617-491-8801



#### CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail, postage prepaid, in an envelope addressed to the Assistant Commissioner for Patents, U.S. Patent and Trademark Office, Box Sequence, P.O. Box 2327, Arlington, Virginia 22202 on the date indicated below.

April 15, 2002

Date

Stephanie L. Leicht

Stephanie L. Leicht



**Copy of Replacement Paragraphs Marked to Show Changes Made  
(Added matter shown by underlining)**

On page 35, at line 6:

For construction of a recombination vector, two linker sequences were inserted into the pBlueScribe vector pBS+ (Stratagene): the first contained restriction sites for *NheI*, *SpeI*, *PacI* and *BglI* followed by a *loxP* sequence (ATAACTTCGTATAGCATACATTATACGAAGTTAT) (SEQ ID NO:6) and was introduced into *PstI*/*XbaI* sites of the vector; the second contained another *loxP* sequence followed by restriction sites *HpaI*, *Clal* and *PmeI* and was introduced into *BamHI*/*Asp718* sites. A gene cassette comprising of a "humanized" version of the ORF coding for GFP (*gfp-h*) under the control of the HCMV enhancer/promoter and the *Ptk*/*PY441* enhancer-driven *neoR* selection marker was excised from plasmid pUF5 (Zolotukhin et al., 1996, J. Virol. 70, 4646-4654) and inserted into the recombination vector via *BglII* sites.

On page 35, at line 18:

At the 5' and 3'-positions of this *loxP*-flanked gene cassette, two HCMV sequences with homology to the gene region containing the open reading frames US9 and US10 were inserted. For this, viral sequences were amplified from template pCM49 (Fleckenstein et al., 1982, Gene 18, 39-46) via PCR in a 35-cycle program (denaturation 45 sec at 95°C, annealing 45 sec at 55°C and elongation 2 min at 72°C) by the use of Vent DNA polymerase (New England Biolabs). A US10-specific sequence of 1983 bp in length was generated using primers US10[200900]*SpeI* (GCTCACTAGTGGCCTAGCCTGGCTCATGGCC) (SEQ ID NO:7) and US10[198918]*PacI* (GTCCTTAATTAAGACGTGGTTGTGGTCACCGAA) (SEQ ID NO:8) and inserted at the vector 5' cloning position via *SpeI*/*PacI* restriction sites (see bold-print). A US9-specific sequence of 2010 bp was generated using primers US9-3'*PmeI* (CTCGGTTTAACGACGTGAGGCGCTCCGTCACC) (SEQ ID NO:9) and US-5' *Clal* (TTGCATCGATACGGTGTGAGATACCACGATG) (SEQ ID NO:10) inserted at the vector 3' cloning position via *PmeI*/*Clal* restriction sites.

At the paragraph bridging pages 35 and 36:

The resulting construct pHM673 was linearized by the use of restriction enzyme *NheI* and transfected into HEF cells via the electroporation method using a Gene Pulser (Bio-rad; 280 V, 960  $\mu$ F, 400  $\Omega$ ). After 24 h of cultivation, cells were used for infection with 1 PFU/ml of HCMV strain AD169. Selection with 200  $\mu$ g/ml G418 was started 24 h post infection. Following 3 weeks of passage in the presence of G418, GFP fluorescence could be detected in most of the infected cells. Plaque assays were performed with infectious culture supernatant on HFF cells and single virus plaques were grown by transfer to fresh HFF cells cultured in 48-well

plates. DNA was isolated from cells of 32 fluorescence-positive wells and confirmed for the presence of recombinant virus by PCR. For this, primers US9[198789] (TGACGCGAGTATTACGTGTC) (SEQ ID NO:11) and US10[199100] (CTCCTCCTGATATGCGGTT) (SEQ ID NO:12) were used resulting in an amplification product of 312 bp for wild-type AD169 virus and approximately 3.5 kb for recombinant virus.